(19) Europäisch s Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 330 629**
A2

(12) # EUROPEAN PATENT APPLICATION

(21) Application number: **89830067.8**

(22) Date of filing: **17.02.89**

(51) Int. Cl.[4]: **C 07 C 129/12**
A 61 K 31/155, A 61 K 31/16

(30) Priority: **24.02.88 IT 4766588**

(43) Date of publication of application:
30.08.89 Bulletin 89/35

(84) Designated Contracting States:
AT BE CH DE ES FR GB GR IT LI LU NL SE

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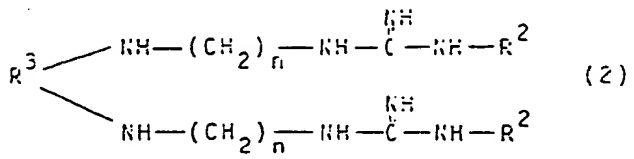
(54) **Guanidine derivatives having hypotensive activity, composition containing them, and process for obtaining them.**

(57) Guanidine derivatives of the following formulas (1) and (2):

$R^1\text{-NH-}(CH_2)\ \ NH\text{-}\overset{\overset{NH}{\|}}{C}\text{-}\ NH\text{-}R^2$ (1)

wherein:
R^1 is hydrogen or optionally substituted cinnamoyl,
R^2 is hydrogen, alkyl or alkenyl, with the proviso that R^1 and R^2 cannot be both hydrogen,
n is an integer from 1 to 8, or:



(2)

wherein:
R^3 is truxinoyl or a truxilloyl each optionally substituted, and R^2 and n are as defined above;
pharmaceutical compositions containing such compounds and a process for their extraction and purification from plant material, in particular from Verbesina caracasana.

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Descripti n

GUANIDINE DERIVATIVES HAVING HYPOTENSIVE ACTIVITY, COMPOSITION CONTAINING THEM AND PROCESS FOR OBTAINING THEM

The present invention relates to new guanidine derivatives having hypotensive activity, to the compositions containing the same and to a process for their production, consisting in extracting and purifying said products from plant materials. More particularly, this invention relates to novel compounds whose formula comprises the guanidine group, which compounds show a remarkable hypotensive activity.

In the field of therapeutical treatment of hypertension some active principles of the class of guanidines are already known, the most widespread among them being guanetidine, whose molecule is made up of an 8-membered heterocyclic saturated ring, containing one nitrogen atom, which nitrogen atom is linked to a chain having two methylene groups and ending in the guanidine group.

Other guanidine compounds known as antihypertensive agents are guanfacine and guanabenz, both characterized by a structure having a benzene ring substituted with two chlorine atoms at one end and having the guanidine group at the other end.

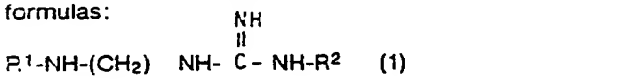
However, all such products are obtained by chemical synthesis, and it is well known to those who are skilled in the art that this feature gives generally higher risks of toxicity with respect to substances of natural origin, because of the possible presence in the final product of trace amounts of reagents, of intermediate compounds and/or reaction by-products. Moreover, it is also well known that drugs obtained by synthesis generally require production processes which are more expensive and more complex than drugs obtained from natural sources.

Accordingly, the object of the present invention is to provide guanidine compounds that show a hypotensive activity comparable to or higher than the activity of compounds already known, but that can be obtained from largely available natural sources through simple and economically convenient extraction and purification procedures.

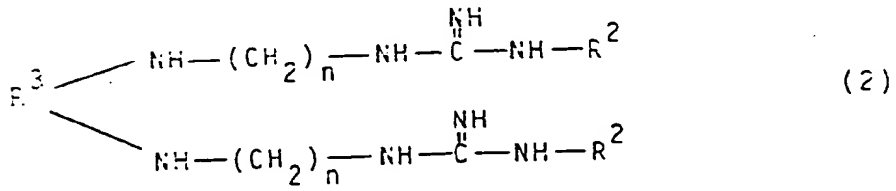
Studies and experimentations carried out to that aim within the present invention resulted in the isolation of a series of guanidine compounds from the extract of a plant, the Verbesina caracasana (Compositae), which compounds showed a remarkable hypotensive activity. Although only the above-mentioned species has been investigated, it cannot be excluded that similar compounds (which are water soluble) can be found in other species of Verbesina, especially if the fact is considered that preceding phytochemical studies reported in the literature for such genus are in all cases limited to the examination of the liposoluble components.

Further to the newly isolated guanidine compounds, a number of structurally related compounds have been synthesized and tested, thereby resulting in the definition of a new class of guanidine derivatives showing remarkable hypotensive activity.

Accordingly, the present invention provides new guanidine derivatives having either of the following general formulas:



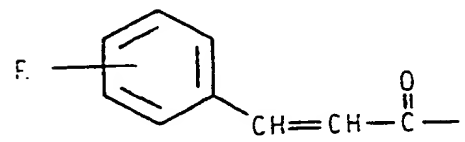
$$R^1\text{-}NH\text{-}(CH_2)\ NH\text{-}\overset{\overset{NH}{\|}}{C}\text{-}NH\text{-}R^2 \quad (1)$$



(2)

wherein:

R^1 is hydrogen or optionally substituted cinnamoyl, i.e.:

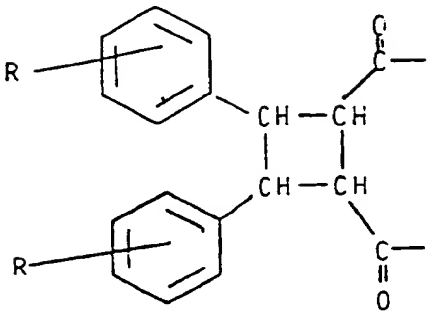


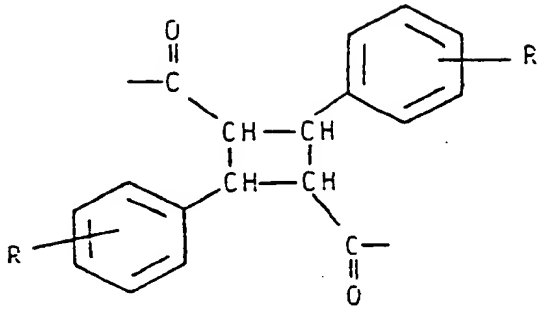
where R represents hydrogen or one or more substituents,

R^2 is hydrogen, alkyl or alkenyl, with the proviso that R^1 and R^2 cannot be both hydrogen, and

n is an integer from 1 to 8, and

R^3 is truxinoyl or truxilloyl, each optionally substituted, i.e., respectively:

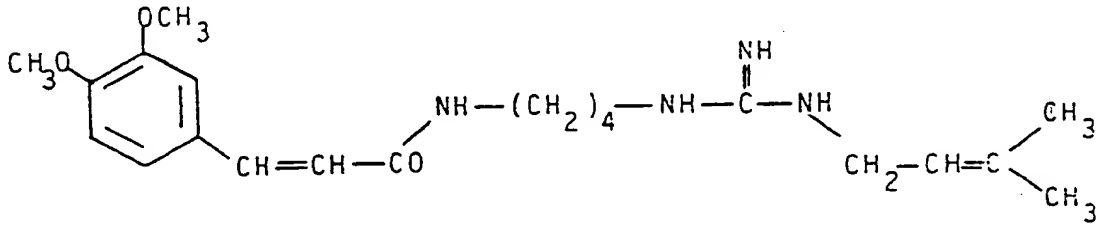




where R has the same meaning as above.

Preferably, the alkyl or alkenyl group R^2 has up to 5 carbon atoms; more preferably, R^2 is prenyl, i.e. 3-methyl-2-butenyl.

More specifically, the invention includes the following four compounds which have been isolated and identified in the extract of Verbesina caracasana:



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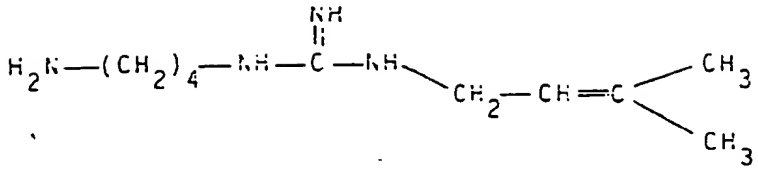

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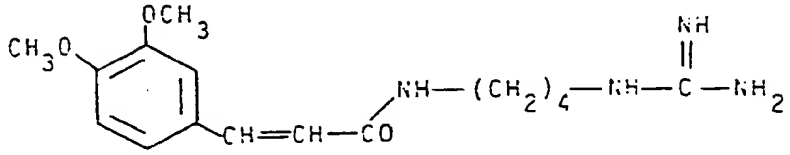

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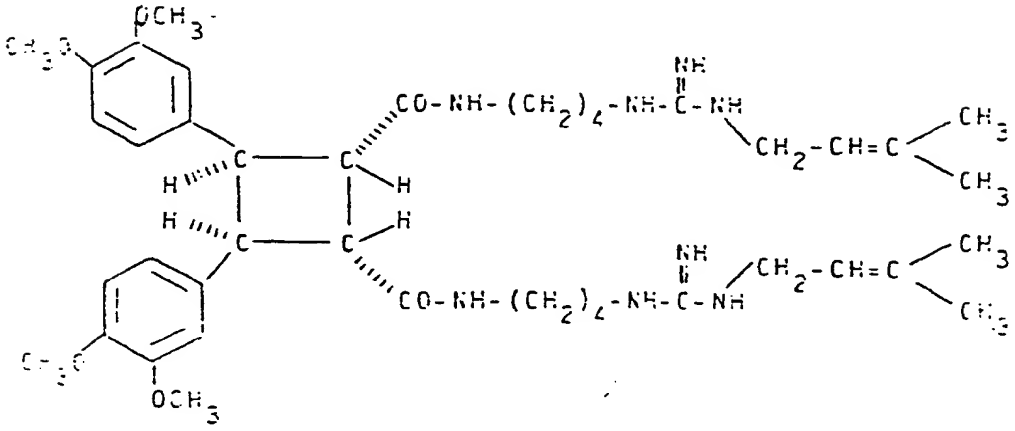


(6)

Compound (3) has been called guanido-I, and is comprised in the general formula (1) for R^1 = 3,4-dimethoxycinnamoyl, n = 4 and R^2 = prenyl;compound (4), called guanido-III, as a matter of practice is a prenylagmatine (agmatine, which is already known, is 4-(aminobutyl)guanidine); compound (5) has been called guanido-V and it can be practically derived from guanido-I without the prenyl radical on the guanidine group; compound (6) comes within the general formula (2) when R^3 is bis(3,4-dimethoxy)β truxinoyl, n is 4, R^2 is prenyl, and it has been called diguanido-II.

It can be easily recognized that compounds (4) and (5) (guanido-III and -V) are fragments of the molecule of the guanido-I, and that diguanido-II has a structure deriving from a dimerization of guanido-I through addition to the double bonds.

Chemical characterization of the preferred compounds of the present invention will be given further on in the example, and the pharmacological data proving the high hypotensive activity of said compounds will also be reported.

The invention also includes pharmaceutical compositions for treating hypertension containing, as the active ingredient, one of the guanidine compounds of the formulas (1) or (2), or a mixture of two or more of said compounds, or even an unfractionated extract of Verbesina, in particular of Verbesina caracasana.

The present invention further provides an extraction and purification process for obtaining the above-mentioned compounds from plant materials, said process being characterized in that it comprises the operations of:

a) treating the plant material, made into small piec s or ground, with an alcoholic solvent for extraction;
b) removing the solv nt in vacuo;
c) distributing the extraction residue between ethyl acetate and water;
d) subjecting the water fraction to lyophilization (freezedrying).

Preferably, in order to increas the purity of the product, the raw lyophilized extract from the last

above-mentioned operation is suspended again in an anhydrous alcoholic solvent and filtered, so as to remove any remaining insoluble material, which is made up mainly of inorganic salts; then the solvent is removed by evaporation, and a new, purified raw extract is obtained. In order to further increase the purity of such product, the preceding operation can be repeated a number of times with decreasing volumes of the alcoholic solvent.

To obtain a higher yield of the compounds of the invention it is also preferable to recover through water extraction the possible part of the active principle left behind in solution in the ethyl acetate employed in step c).

As an alternative to the process disclosed above, it is possible to substitute a water extraction at room temperature for the steps a), b) and c), with substantially similar yields.

The raw extract resulting from step d) (or the purified product obtained according to the preferred procedure) is then subjected to a process for separating the components on a silica column, eluting with chloroform containing increasing amounts of methanol; said separation can also be performed employing Sephadex LH-20 and eluting with methanol.

In addition to the extraction process proposed above, which by the moment represents a simple and economic way for producing the guanidine compounds of the invention, it is also possible to provide other production techniques, such as for instance biotechnological processes from plant cell cultures, seedlings, immobilized cells or even from microorganisms (bacteria, yeasts).

Furthermore, the compounds of the invention may be prepared by chemical synthesis, by adapting methods already known, for instance as described in some of the following examples.

The present invention will be now disclosed, for illustrative but not for limitative purposes, with particular reference to some of its preferred embodiments, in the following examples.

EXAMPLE 1

Extraction procedure

10 kg of Verbesina caracasana (the whole fresh plant) collected near Valencia (Venezuela, Carabobo State) is grossly made into pieces and extracted by a continuous process (with a Soxhlet apparatus) for 15-20 hours with methanol. After removing the solvent in vacuo at 30-35°C, the residue (about 700 g) is distributed between ethyl acetate and water.

Such operation allows to eliminate a large part of undesired compounds (triterpenes, chlorophyll, and so on), which are left behind in the organic solvent. The main part of the desired compounds are separated in the water phase (fraction A). The remaining part of the active ingredients, extracted by ethyl acetate, is recovered by a further water extraction (fraction B). The water soluble portion (A + B) is concentrated in vacuo at 35-40°C and lyophilized, giving a raw extract C (of about 380-400 g).

By substituting ethanol for methanol in the continuous extraction process (Soxhlet), or by extracting the plant with water at room temperature, substantially the same yields of the raw extract C are obtained.

Then, the raw extract C is suspended in absolute methanol or ethanol and filtered. Such operation, as already illustrated above, results in the elimination of a large part of inorganic salts (about 70 g), the presence of which would complicate the subsequent chromatographic separation process. A further evaporation of the alcohol soluble portion gives the raw extract (310-330 g). By repeating the operation with lower amounts of alcohol further amounts of salts can be removed.

EXAMPLE 2

Separation of components

Method A

A portion (about 80 g) of the extract D mentioned in the preceding example (it is to be pointed out, however, that the process can be also carried out on the raw extract C, even though the results obtained are not as satisfying) is dissolved/suspended in methanol and then it is absorbed on 100-150 g of silica. After removing the solvent, the mixture (silica + the raw extract D) is put at the top of a silica column prepared in chloroform. Eluting with 3.5 l of chloroform allows elimination of trace amounts of chlorophyll and other undersired compounds. Repeated luting with chloroform mixtures containing increasing amounts (10-50%) of methanol gives the compounds diguanido-II, guanido-I, - III, -IV (unidentified) -V, agmatine and galegine (a compound which is already known and definable as prenylguanidine) as relatively pure substance. The approximate amounts of each compound which are obtain d from about 80 g of the raw extract D are the following: guanido-I: 3-6 g; diguanido-II: 3-5 g; guanido-III: 0.5-1 g; guanido-IV: 1-2 g; guanido-V: 0.5-1 g; agmatine: 0.5-1

g.

The yield of ach component depends on various factors such as for instance the harvesting time or the ways by which the raw extract D is prepared. It has been observed that harvesting the plants at the end of summer gives higher yields of diguanido-II with less amounts of guanido-I, probably owing to photodimerization of the latter. Similarly, prolonged heating or the employment of higher temperatures during evaporation result in higher yields of guanido-III and -IV, again with less amounts of guanido-I, probably owing to partial hydrolysis of the latter.

It is to be observed that, if the above-mentioned compounds are to be obtained in their pure form, it is necessary to perform a further chromatographic purification.

Method B

The raw extract of Verbesina caracasana has also been subjected to a preliminary purification process by Sephadex LH-20. By eluting with methanol a partial separation into three fractions according to the molecular weight can be obtained. The first fraction contains the compound diguanido-II, while the second fraction consists of a mixture of the compounds guanido-I - IV and -V, and the third fraction is made up of a mixture of guanido- III, agmatine and galegine. The purification by a Sephadex LH-20 column was also employed to obtain the individual components in their pure form.

EXAMPLE 3

Chemical characterization of guanido-I

The structure attributed according to formula (3) has been determined on the basis of spectroscopic data and of the results obtained by alkaline hydrolysis.

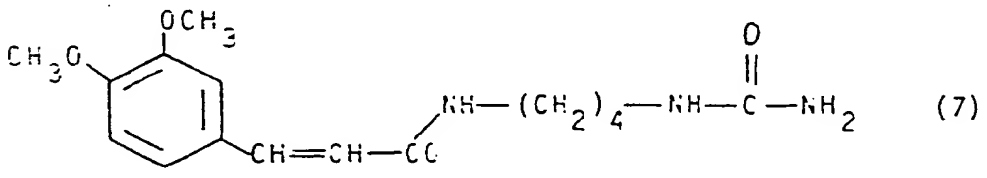
Mass spectrum at high resolving power: M^+ 388.2472; calculated for $C_{21}H_{32}O_3N_4$, M^+ 388.2474. Mass spectrum: 388 (6), 387 (5), 373 (3), 302 (5), 301 (12), 207 (45), 206 (32), 191 (100), 163 (21), 133 (11), 91 (45).

UV spectrum (MeOH), 291 and 330 nm. ^{1}H NMR spectrum (D_2O; cis-form predominant), δ ,7.15-6.95 (3H, m, H-2. H-5, H-6); 6.78 (1H, t, J = 7 Hz, CH=),6.52 (1H, d, J=13 Hz, H_α). 5.98 (1H, d, J=13 Hz, H_β), 5.18 (1H, broad t, J=7 Hz, HC=C), 3.85 and 3.82 (3H each, 2s, 2x OMe), 3.74 (2H, t, J = 7 Hz, CH_2), 3.5-2.9 (4H, m, 2x CH_2), 1.76 (6H, broad s, 2x Me), 1.6-1.3 (4H, m, 2x CH_2).

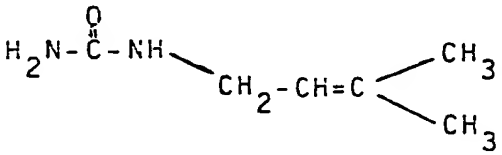
^{1}H NMR spectrum ($CDCl_3$-CD_3OD, 3-1; 1/1 mixture of the cis-and trans-forms), δ, 7.55-6.90 (3H, m, H-2, H-5. H-6), 7.52 (1/2H, d, J = 16 Hz, trans H-α , 6.71 (1/2H, d, J = 13 Hz, cis H-α 6.62 (1/2H, d, J = 16 Hz, trans H-β . 5.96 (1/2H, d, J = 13 Hz, cis H-β , 5.25 (1H, broad t, J = 7 Hz, CH=), 3.75 (6H, s, 2x OMe), 3.73 (2H, d, J = 7 Hz), 3.5-3.0 (4H, m, 2x CH_2), 1.75 (6H, broad s, 2x Me), 1.75-1.35 (4H, m, 2x CH_2).

^{13}C NMR spectrum (CD_3OD), δ, 170.1, 169.1 (CO), 157.4 (C=NH), 152.3, 150.8 (C-3, C-4), 141.7, 140.8, (C-α), 139, 138.2 (C-1), 129.3 (C=), 124.6, 122.6 (C-6), 123.3 (CH=), 119.7, 119.4 (C-β), 114.3, 112.9 (C-2), 112.4. 111.6 (C-2), 56.5 (2x OMe), 42.3, 40.5, 39.8 (3x N-CH_2), 27.7, 27.2 (2x CH_2), 25.8, 18.1 (2x CH_3).

The compound (3) gave by hydrolysis (with 0.25 N $Ba(OH)_2$ under reflux for 4 hours) a mixture of the cis- and trans-3,4-dimethoxycinnamic acid and the following compounds (7) - (9) reported herein below:



(7)



(8)

$H_2H-(CH_2)_4-NH-\overset{\overset{O}{\|}}{C}-NH_2$ (9)

Compound (7): melting point 200-1°C. $C_{16}H_{23}N_3O_4$: calculated 321.1688; M^+, 321.1673 (HR MS). The results of the UV, IR, ^1H NMR spectra as well as the mass spectrum fragmentation are in agreement with the structure.

Compound (9): melting point 102-3°C; $C_5H_{13}N_3O$ (M.W. 131), $[M + H]^+$ 132 (MS, chemical ionization); ^{13}C NMR (D_2O) 168 (C=O), 39.9 and 39.8 (N-CH_2 x 2), 26.8 and 24.8 (CH_2 x 2); ^1H NMR and mass spectrum fragmentation agree with the structure.

EXAMPLE 4

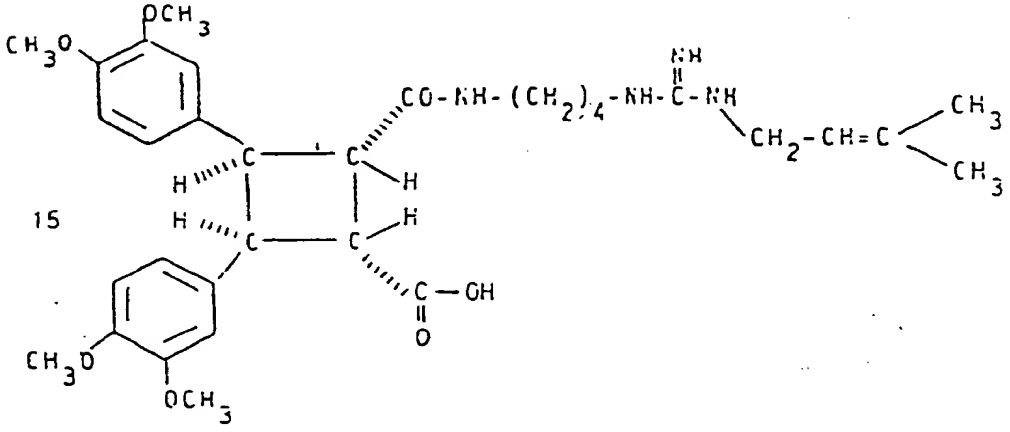
Chemical characterization of diguanido-II

The structure assigned to the compound according to the formula (6) has been determined on the basis of spectroscopic data and of the results of alkaline and acid hydrolyses.

$C_{42}H_{64}N_8O_6$ (M.W. 776), mass spectrum (FAB), 777 $[M + 1]^+$ (100), 761 (4), 747 (6), 709 (11), 667 (2), 650 (3), 636 (5), 622 (2), 596 (3), 579 (25), 551 (11), 389 (46), 191 (57). ^1H NMR spectrum ($CDCl_3$-CD_3OD, 3-1): δ, 7.3 (2 x 1H, s), 6.7-6.4 (2x 2H, m), 5.2 (2x CH, broad t), 4.4-3.5 (2x 8H, m), 3.73 (2x 3H, s), 3.62 (2x 3H, s), 3.3-3.0 (2x 4H, m), 1.70 (2x 6H, broad s).

^{13}C NMR spectrum (CD_3OD): δ, 174.8 (CO), 157.8 (C=NH), 150.4, 149.4 (C-3, C-4), 134.3 (C=), 130.9 (C-1), 122.0, 120.0 (C-6, CH=), 114.2, 113.0 (C-5, C-2), 57.0, 56.9 (2x OMe), 46.4, 45.6 (2x CH), 42.9, 41.1, 40.4 (3x N-CH_2), 28.1, 27.8 (2x CH_2), 26.4, 18.7 (2x CH_3).

The compound (6) gave by hydrolysis (with 2N NaOH, 5 days at room temperature) the compounds (4) (guanido-III), (8) and (10), the last one reported hereinbelow:

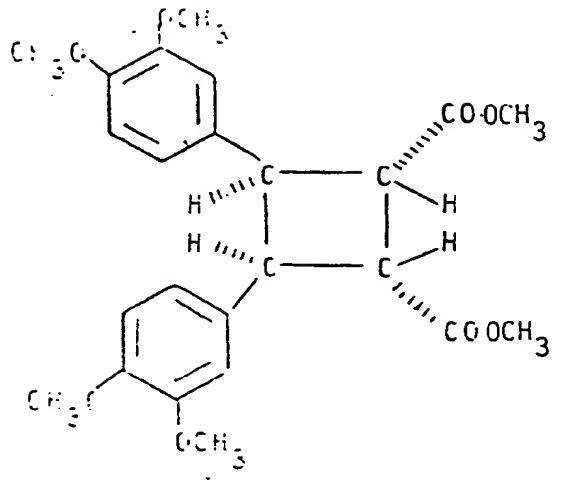


(10)

Compound (10): ^1H NMR ($CDCl_3$-CD_3OD); δ, 6.85 (2x 2H, broad s), 6.45 (2x 1H, broad s), 5.2 (1x C=, broad t), 4.4-4.2 (2x CH, m), 3.9-2.8 (12H, m), 3.70 (2x OMe, s), 3.6 (2x OMe, s), 1.75 (6H, broad s).

^{13}C NMR ($CDCl_3$-CD_3OD); δ 179.0, 173.7 (CO), 158 (C=NH), 148.0, 147.9, 146.8, 146.6 (C-3, C-4), 138.3 (C=), 133.2, 132.8, (C-1), 119.7, 119.6, 118.5 (C-6, C=) 118.8, 111.5, 110.5, 110.4 (C-5, C-2), 55.3, 55.2 (4x OMe), 46.7, 45.3, 44.3, 40.7 (4x CH), 42.9, 39.1, 38.1 (3x N-CH_2), 26.2, 24.8 (2x CH_2), 25, 17.3 (2x CH_3).

The compound (10) gave by acid hydrolysis (with HCl 2N in MeOH, under reflux for 3 hours) the following compound (11):



(11)

Ccmpound (11): ^{1}H NMR ($CDCl_3$); δ, 6.67 (2x 1H, d), 6.57 (2x 1H, d), 6.22 (2x 1H, d), 4.30 (2x 1H, d), 3.79 (2x 3H, s), 3.77 (2x 1H, d), 3.75 (2x 3H, s), 3.63 (2x 3H, s).

^{13}C RMN (acetone-d_6); δ, 173.7 (C=O), 151.4, 148.8 (C-3', C-4'), 132.6 (C-1'), 120.8 (C-6'), 113.2 (C-2'), 112.0 (C-5'), 55.91, 55.89 (OMe), 52.1 (COOMe), 45.6 (C-β), 43.9 (C-α)

Mass spectrum: M^+ 444, m/z 300.

EXAMPLE 5

Chemical characterization of guanido-III

The structure assigned to the compound according to formula (4) has been determined on the basis of spectroscopic data and of the results of alkaline hydrolysis.

$C_{10}H_{22}N_4$ (M.W. 198), mass spectrum (FAB) 199 $[M+1]^+$

^{1}H NMR spectrum ($CDCl_3$-CD_3OD, 3-1): δ, 5.2 (1H, m, =CH), 4.0-3.60 (10H, m, 5x CH_2), 1.75 (6H, s, 2x CH_3).

^{13}C spectrum (4 • AcOH) (CD_3OD): δ, 181 (CD, AcOH) 155.6 (C=NH), 139 (C=), 117.5 (CH=), 40.5, 39.1, 39.0 (3x N-CH_2), 25.1, 24.1 (2x CH_2), 24.7 (CH_3, AcOH), 23.3, 17.1 (2x CH_3).

The compound (4) gave by hydrolysis (with 0.25 N $Na(OH)_2$, under reflux for 2 hours) the compounds (8) and (9) (already dis closed in Example 3) and urea.

EXAMPLE 6

Chemical characterization of guanido-V

The structure assigned to the compound according to formula (5) has been determined on the basis of spectroscopic data and of the results of mild hydrolysis.

$C_{16}H_{24}N_4O_3$ (M.W. 320), mass spectrum (FAB) 321 $[M+1]^+$.

^{1}H NMR (CD_3OD): δ, 7.5 (H-2), 7.2 (H-6), 7.0 (H-5), 6.8 (H_α), 6.1 (H_β), 4.0 (2x OMe), 3.55-3.3 (2x CH_2), 1.9-1.7 (2x CH_2).

^{13}C NMR (CD_3OD): δ, 169.2 (CO), 157 (C=NH), 150, 148.9 (C-3, C-4), 141.3 (C-α), 128.7 (C-1), 122.2 (C-β), 121.9 (C-6), 113.2, 111.3 (C-5, C-2), 55.4, 55.3 (2x OMe), 39.8, 39 (2x N-CH_2), 26.8, 26.3 (2x CH_2).

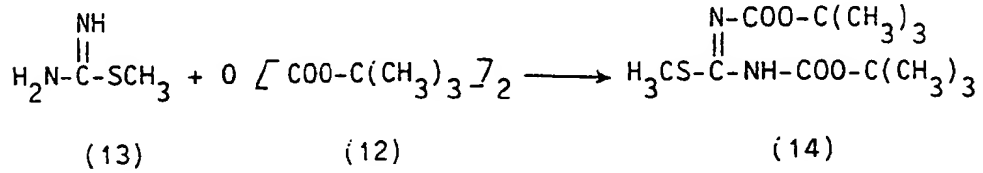
The compound (5) gave by hydrolysis (with 0.25 N $Na(OH)_2$, under reflux for 2 hours) a mixture of the cis- and trans-3,4-dimethoxycinnamic acids and the compounds (7) and (9) already disclosed in example 3.

EXAMPLE 7

Chemical synthesis of guanido-V, i.e. 4-[(3,4-dimethoxycinnamoyl)amino]butylguanidine

5.8 g of di-tert-butyl dicarbonate (12) is added to 2.5 g of S-methoxythiourea H_2SO_4 (i.e. 2-methyl-2-thiopseudour a sulfat) (13) and the mixture is left in a two-phase CH_2Cl_2-$NaHCO_3$ system (50 ml + 50 ml, respectively) for two days under stirring, so as to achiev the tert-butoxycarbonylation (t-BOC) of the two amine groups.

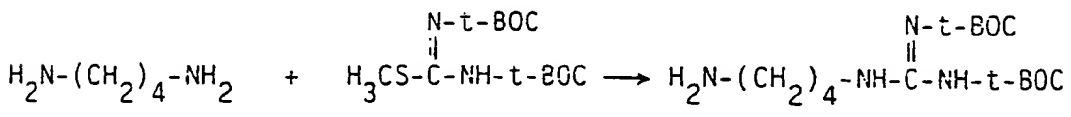
The scheme of reaction is as follows:



(13) (12) (14)

After separation of the two phases, the water fraction is further extracted with CH_2Cl_2. After purification, 3.4 g of compound (14) is obtained.

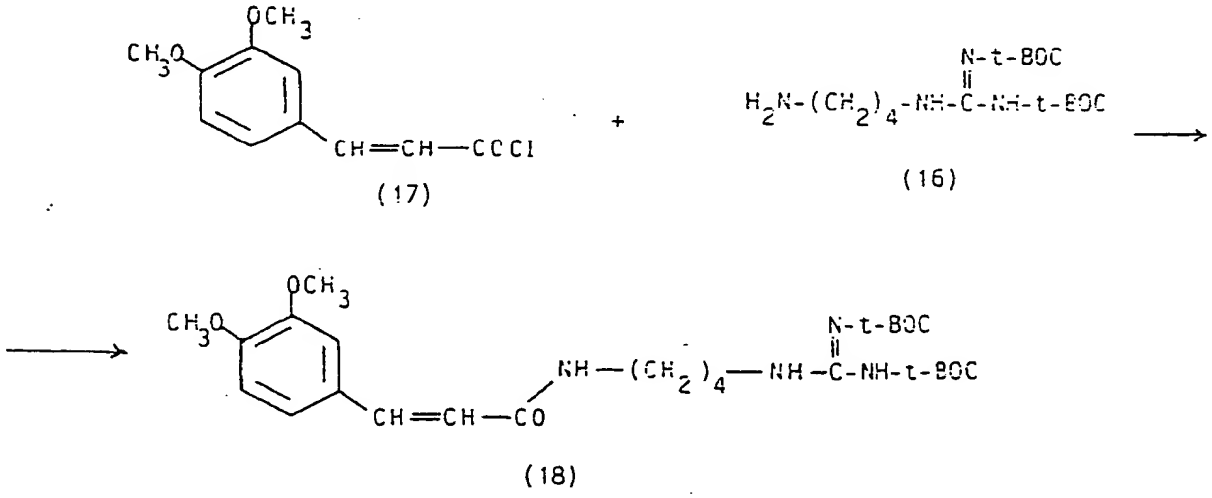
200 mg of compound (14) is reacted with 63 mg of 1,4-diaminobutane (tethramethylenediamine) (15) in 5 ml THF and 0,1 ml H_2O. The reaction mixture is heated to 50°C for 3 hours, so that the following reaction occurs:



(15) (14) (16)

After evaporation of the solvent, the residue is washed with $NaHCO_3$ 5% and extracted with $CHCl_3$. After purification on silica 120 mg of compound (16) is obtained.

The compound (16) (500 mg) is reacted with 370 mg of 3,4-methoxycinnamoylchloride (17) in THF-DMF (5 ml - 5 ml) for 16 hours at room temperature, according to the following scheme:



(17) (16) (18)

After purification on silica, by eluting with ethylacetate-hexane (10-90), 300 mg of compound (18) is obtained.

Thereafter, 1,5 ml of TFA is added to 23 mg of compound (18) and the mixture is stirred at room temperature for 43 minutes. Then, the mixture is dried over P_2O_5 and purified on Sephadex LH-20, by eluting with methanol, thus obtaining 12 mg of guanido-V, i.e. compound (5).

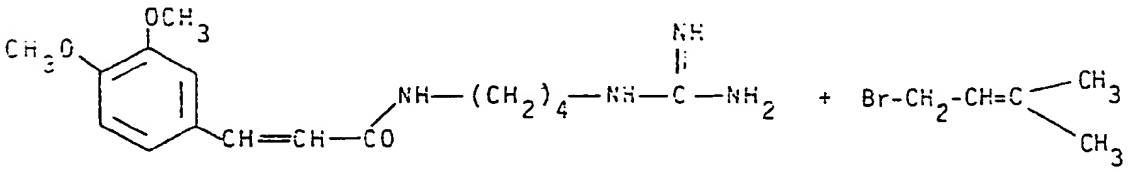
EXAMPLE 8

Chemical synthesis of guanido-I, ie. 1-{4-[(3,4-dimethoxycinnamoyl)amino/butyl} -3-prenylguanidine

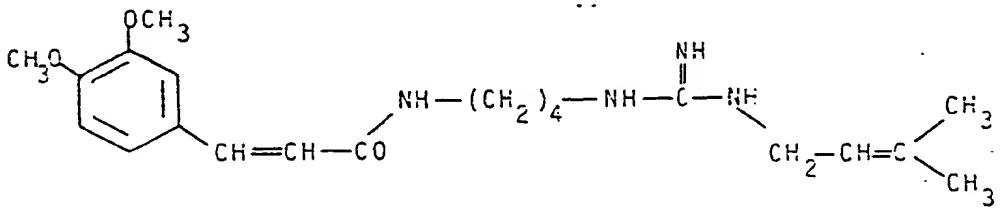
1,5 g of prenyl bromide (γ,γ'-dimethylallylbromide) (19) is added to a solution containing 1 g of guanido-V (obtained, f.i., according to the procedure of Example 7), i.e. compound (5), in 10 ml of anhydrous THF, and a catalytically effective amount of N,N'-dimethylaminopyridine.

The reaction mixture is kept under stirring at room temperature for 6 hours. After purification, 750 mg of compound (3), i.e. guanido-I, is obtained.

The scheme of the reaction is the following:



(5) (19)



(3)

EXAMPLE 9

Chemical synthesis of 3-[(3,4-dimethoxycinnamoyl)amino]propylguanidine

The same procedure as in example 7 is followed, with the exception that 1,3-diaminopropane is employed instead of compound (15) (1,4-diaminobutane).

EXAMPLE 10

Chemical synthesis of 1-{3-[(3,4-dim thoxycinnamoyl)amino]propyl}-3-prenylguanidine

In the same manner as described in Example 8, the title compound is obtained by alkylation of the compound of Example 9.

EXAMPLE 11

Ch mical synthesis of 6-[(3,4-dimethoxycinnamoyl)amino]hexylguanidine

The procedure described in Example 7 is repeated employing hexamethylenediamine (1,6-hexanediamine) instead of compound (15).

EXAMPLE 12

Chemical synthesis of 1-[6-[(3,4-dimethoxycinnamoyl)amino]hexyl]-3-prenylguanidine

The procedure described in Example 8 is repeated starting from the compound of Example 11.

Activity of the raw extract

In order to check the activity of the raw extract of Verbesina carasana, a 0.9 % sodium chloride solution of such extract was administered by i.v. "rapid" route to dogs under general chloralose anaesthesia. The responses induced at the level of (mean) systemic arterial pressure, respiratory frequency and following bilateral carotid occlusion were evaluated. The results, which are reported in the following Table 1, put into evidence that the hypotensive activity is proportional to the dose, the maximum value of the hypotensive activity being reached at the dose of 2 mg/kg.

TABLE 1

NUMBER OF ANIMALS	DOSE (mg/kg)	BP (mmHg)	- Δ p (mmHg)	- Δ p (%)
5	0.5	162±10	23±10	13
6	1	154±8	48±18	31
6	2	159±7	92±14	58
4	4	155±6	85±8	55

BP = aterial basal pressure

-Δ p= decrease in pressure

The administration of the extract to mice causes hair erection, stimulation, and subsequently death from respiratory block.

Activity of the compounds of the invention

Guanido-l

In order to test its activity, guanido-l (compound (3)), dissolved in a 0.9 % sodium chloride solution, was administered through i.v. "rapid" route to male Wistar rats anaesthetised with 10 % ethyl urethan (1 ml/hg).

The parameters estimated and reported in the following Table 2 for various doses of guanido-l administered are: changes in the systolic and diastolic systemic arterial pressure (ΔAP), changes in the heart rate (ΔHR), in the maximum rate of increase in the left ventricular isovolumetric pressure (Δdp/dt) as an index of cardiac inotropism and changes in the respiratory frequency (ΔRF)

TABLE 2

No. of animals	Dose (µg/kg)	Δ AP (mmHg) SYST	Δ AP (mmHg) DIAST	Δ HR (beats/min)	Δ dp/dt (mmHg/sec)	Δ RF (acts/min)
6	50	-4±2	-5±1	+8±3	+52±8	+6±2
6	100	-6±2	-6±2	+10±1	+141±25	+9±4
6	200	-11± 3	-10±2	+13±2	+1284±78	+12±2
6	400	-15±4	-14±3	+14±5	+1712±107	+16±3
12	800	-17±3	-15±3	+18±4	+2140±263	+25±6
12	1600	-21±6	-18±4	+22±3	+2996±104	+23±9
12	3200	-25±5	-29±4	+25±8	+2984±362	+26±7
12	6400	-34±4	-29±2	+20±4	+3074±441	+29±5

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The results reported in Table 2 point out that guanido-I low rs the value of AP according to a linear dependence on the dose, whereas it causes an increase in the values of HR, dp/dt and RF. In the case of the tidal volume (TV), a linear increase has been observed with the doses of guanido-I administered. Such cardiovascular effects do not seem to be correlated to significative actions of the active ingredient at the level of the peripheral adrenergic r ceptors (α_1 - α_2; β_1 - β_2), rather to central neurogenic mechanisms (as, for instance, it can be put int evidenc by exp riments of spinalization and of ganglionic block) and to peripheral-effectorial mechanisms, for example on the processes of muscular contractility, in myocardium cells and vascular myocells.

In particular, the heart rate (HR) increases at all doses tested owing to a direct effect (at the cardiac and/or central level) and to a reflection following arterial hypotension. Similar considerations also hold true for dp/dt, while the increase in the respiratory frequency (RF), correlated to the doses of the compound, and present at all doses tested, can be traced to a constant stimulation effect on the central respiratory centers.

The lethal dose 50 (LD_{50}) of guanido-I through i.p. route in mice turned out to be 57 mg ± 3.6 per kg.

Diguanido-II

In order to check its activity, diguanido-II was dissolved in a 0.9 % sodium chloride solution and administered through "rapid" i.v. route in male Wistar rats, under general anaesthesia obtained by means of 10 % ethyl urethan (1 ml/hg).

The results obtained with various doses of the compound are reported in Table 3, in which the changes in the aortic flow at the iliac bifurcation (/ AF) as well as the changes in the stroke volume (/ SV) are reported in addition to the parameters already illustrated above.

Table 4 also shows the percentage values corresponding to those shown in Table 3 and, in addition, it also shows the values of the percentage changes in the tidal volume (Δ TV).

TABLE 3

NO. OF ANIMALS	DOSE (µg/kg)	Δ AP (mmHg)		Δ HR (beats/min)	Δ dp/dt (mmHg/sec)	Δ AF (ml/min)	Δ SV (µl)	Δ RF (acts/min)
		SYST	DIAST					
12	50	+17.2±2.4	+9.8±1.7	-9±3	+1070±90	+18.8±3.40	+72.6±5	+5.3±2.7
12	100	+11.7±2.5	+10.2±1.1	-10±1	+1129±82	+10.8±1.20	+19.8±6	+6.4±2.93
12	200	-14.4±1.4	-13.1±1.8	-13±4	+1369±170	-13.8±4.20	+23.1±6	+8.6±3.7
18	400	-19.8±1.9	-17.0±1.4	-16±5	+2615±181	-14.3±5.7	-46.2±4	-3.1±0.9
18	800	-30.0±3.6	-31.2±3.1	-16±5.5	+3282±269	-25.0±6.1	-85.8±9	-14.6±4.3
18	1600	-41.1±5.3	-43.4±6.8	-30±6	+3676±264	-36.1±4.8	-97.4±8	-61±8.9
18	3200	-47.8±6.5	-53.0±4.2	-41±8	+4708 ±430	-41.0±6.9	-117.2±8	-67±2.9

TABLE 4

No. OF ANIMALS	DOSE (µg/kg)	Δ AP%		Δ HR%	Δ dp/dt%	Δ AF%	Δ SV%	Δ RF%	Δ TV%
		SYST	DIAST						
12	50	+15.0±2.7	+11.0±1.7	-3±1	+17±4	+43±3	+61±5	+6±3	+13±3
12	100	+10.0±2.6	+11.0±1.1	-3±1	+18±1	+25 ±1	+17±6	+7±3	+4±1
12	200	-13.0±1.4	-9.0±1.8	-4±0.3	+21±4	-32±4	+19±6	+9±4	+3±2
18	400	-18.0±1.9	-18.0±1.4	-5±2	+41±5	-33±6	-39±4	-3±1	-51±7
18	800	-27.0±3.6	-34.0±3.1	-5±2	+51±7	-57±6	-72±9	-15±4	-93±8
18	1600	-36.0±5.3	-47.0±6.8	-9±3	+57±6	-83±5	-82±8	-63±9	-98±5
18	3200	-42.0±6.5	-58.0±4.2	-12±3	+73±9	-94±7	-98±8	-70±3	-100

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The compound diguanido-II gives biphasic effects on AP, AF, RF and TV: an increase in such parameters at doses between 50 and 100 µg/kg (AP, AF) or 50 and 200 µg/kg (SV), RF and TV), and a decrease in the same paramet rs a higher doses (up to 3200 µg/kg).

Previous treatment with diguanido-II decreased the cardiovascular responses induced by hypertensive doses of nor-adrenaline and of adrenaline, and it increased the cardiovascular responses to administration, through i.v. route, of acetylcholine and isoproterenol, according to a linear law with respect to the doses of the agonists in question.

Previous treatment with Hexameton (ganglioplegic) caused an increase in the cardiovascular responses to diguanido-II as mentioned above, whereas vagotonism was not followed by any modification in the response to diguanido-II.

Reserpinization, spinalization and previous treatment with alpha-blocking agents were not efficient in modifying cardiovascular and respiratory responses to diguanido-II.

As a matter of practice, the increase in RF and TV at lower doses can be ascribed to a central stimulation effect on the respiratory centers. A central effect of the compound is also acting in the case of cardiovascular responses. At higher doses, a depression effect occurs, on the contrary, on the respiratory centers, which effect is not related to the cardiovascular ones. Indeed, such effects are to be ascribed to a reduction of the peripheral vascular resistance.

The reduction of heart rate (HR), at all doses of the compound, cannot be ascribed to reflexion mechanisms (baroreceptorial mechanisms). Such reduction persists in the presence of a constant increase in dp/dt and of inversion of the AF, SV and RF responses. It is thus evident that bradycardia induced by the compound guanido-II can only be explained by central mechanisms, differently with respect to the effects developed by the guanido-I.

The lethal dose 50 (LD_{50}) of the diguanido-II, through i.p. route in mice, turned out to be 7 mg ± 0.6 per kg.

Guanido-III, -IV, -V, agmatine and galegine

The active ingredients called guanido-III, -IV, -V, as well as agmatine and galegine, were also tested, after having been administered through "rapid" i.v. route in male Wistar rats (weighing average 280 ± 5 g) under general anaesthesia obtained by means of 10% ethyl uretan (1 ml/hg, i.p.). The general experimental conditions were exactly the same as previously mentioned for guanido-I and diguanido-II, so that the cardiovascular and respiratory effects could be compared.

The parameters measured are as follows: systolic and diastolic systemic arterial pressure (AP), heart rate (HR), maximum rate of increase in the left ventricular isovolumetric pressure (dp/dt), respiratory frequency (RF) and tidal volume (TV). The doses (in µg/kg body weight, referred to the active substance) varied from 50 µg/kg to 6400 µg/kg (ratio = 2.0); in some cases higher does have been employed.

The experimental data relevant to guanido-III, guanido-IV, guanido-V, agmatine and galegine are shown respectively in Tables 5 through 9.

TABLE 5

Dose (µg/kg)	Δ AP (mmHg) SYST	Δ AP (mmHg) DIAST	Δ HR (beats/min)	Δ dp/dt (mmHg/sec)	Δ RF (%)	Δ TV (%)
200	-2±0.3	-2±0.2	0	+214±20	+7±4	+9±2
400	-3.5±1.5	-2±0.6	0	+321±17	+7±3	+17±6
800	-11±1	-13±4	-15±3	+1356±428	+10±4	+33±9
1600	-13±2	-21±4	-23±10	+2157±655	+18±3	+21±7
3200	-36±7	-36±6	-26±8	+2640±634	-42±9	-73±18
6400	-112±3	-85±3	-310±25	-5422±514	-100	-100

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TABLE 6

Dose (µg/kg)	Δ AP (mmHg) SYST	DIAST	Δ HR (beats/min)	Δ dp/dt (mmHg/sec)	Δ RF (%)	Δ TV (%)
200	-5±2	-5±1	-9±4	+420±72	+40±7	+20±5
400	-9±4	-10±3	-11±7	+1070±148	+56±5	+39±5
800	-11±4	-13±2	-14±7	+1284±201	+16±3	+41±8
1600	-12±5	-16±2	-14±5	+1369±322	+ 2±1	+29±4
3200	-16±3	-24±5	-17±8	+2140±240	-23±8	-61±5
6400	-38±6	-41±6	-59±11	+2354±177	-82±8	-98±2

TABLE 7

Dose (µg/kg)	Δ AP (mmHg) SYST	Δ AP (mmHg) DIAST	Δ HR (beats/min)	Δ dp/dt (mmHg/sec)	Δ RF (%)	Δ TV (%)
200	0	0	0	-392±21	-7±2	-14±4
400	-4±2	-3±1	0	-384±39	-3±	-13±2
800	-5±2	-6±2	0	-430±43	-3±2	+12±4
1600	-8±3	-6±2	0	-642±76	-4±1	+11±3
3200	-10±4	-9±3	0	+684±38	+1±1	+20±6
6400	-11±3	-15±4	-14±6	+2354±214	+10±4	+22±7
12800	-23±5	-28±4	-49±8	+2996±170	+11±3	+74±8
102400	-24±4	-30±5	-67±11	+3040±224	+6±4	+79±12

TABLE 8

Dose (µg/kg)	Δ AP (mmHg) SYST	DIAST	Δ HR (beats/min)	Δ dp/dt (mmHg/sec)	Δ RF (%)	Δ TV (%)
200	-5±2	-4±1	0	+300±89	+9±3	+3±1
400	-6±1	-6±2	0	+334±54	+11±4	+12±4
800	-8±2	-8±2	0	+947±131	+12±4	+14±2
1600	-9±3	-10±1	0	+981±94	+11±2	+25±5
3200	-9±2	-11±2	-7±2	+1356±164	+13±4	+27±4
6400	-11±4	-13±4	-13±5	+3351±417	+13±5	+30±7
12800	-12±2	-18±5	-17±2	+2568±432	+15±2	+31±7
25600	-12±3	-23±4	-17±7	+3565±813	+24±4	+33±9
51200	-15±1	-34±6	-22±1	+5491±869	+31±7	+35±6

TABLE 9

Dose (µg/kg)	Δ AP (mmHg) SYST	DIAST	Δ HR (beats/min)	Δ dp/dt (mmHg/sec)	Δ RF (%)	Δ TV (%)
50	-18±1	-19±3	-32±6	+1498±131	+38±4	+43±4
100	-22±2	-28±6	-46±7	+1712±90	+68±4	+63±5
200	-23±5	-29±5	-94±6	+1797±178	+69±7	+64±7
400	-25±6	-26±4	-107±4	+2568±314	+76±9	+87±9
800	-27±4	-26±4	-106±11	+1926±223	+70±8	+31±3
1600	-22±4	-25±4	-85±5	+1724±104	+133±14	+55±6
3200	-22±3	-36±4	-98±16	+2996±202	+85±6	+40±7
6400	-18±4	-36±2	-42±4	+4494±371	+37±11	+29±8
12800	-22±2.5	-42±3	-15±7	+6420±417	+23±7	+25±5
25600	-19±3	-30±9	-21±6	+6471±274	-34±12	-57±8
51200	-80±8	-50±7	-298±19	-4708±312	-100	-100

The compound guanido-III (Table 5) caused systolic and diastolic arterial hypotension (well evident starting from th dose of 800 µg/kg), bradycardia, incr ase of dp/dt (up to th dose of 3200 µg/kg), increas of RF and TV (up t th dose of 1600 µg/kg). RF and TV were lowered by a dose of 3200 µg/kg; th dose of 6400 µg/kg caused death of the tested animals: after a primary respiratory block, arterial hypotension followed, and then increasing reduction of HR and dp/dt; TV was interested prior than RF.

The compound guanido-IV (Table 6), tested in doses of 50-6400 µg/kg, showed effects similar guanido-III, though with a lower power: systolic and diastolic arterial hypotension, bradycardia, increase of dp/dt; RF and TV increased up to the dose of 1600 µg/kg, and decreased for higher doses; the maximum dose tested did in no case result in death of the animal.

The compound guanido-V (Table 7) caused systolic and diastolic arterial hypotension, more evident from the dose of 3200 µg/kg on; bradycardia was observed starting from the dose of 6400 µg/kg; a slight reduction of dp/dt and RF appeared up to the highest doses; TV showed a similar trend.

The changes of AP and HR induced by agmatine (Table 8) were analogous to those induced by guanido-V; moreover, said active ingredient caused a constant increase in dp/dt, RF and TV.

Galegine (Table 9) caused systolic and diastolic arterial hypotension and bradycardia yet from the dose of 50 µg/kg with a constant increase of dp/dt, RF and TV. Only extremely high doses (25600 and 51200 ug/kg) caused a primary respiratory depression (reduction of RF and TV) and a subsequent arterial hypotension, bradycardia and decrease in the cardiac inotropism (dp/dt), with death of the tested animals.

Comparison with guanidine

The administration of guanidine (50-800 µg/kg) by "rapid" i.v. route, for comparison with the above-described active principles, did not cause in the rats under test significative alterations of RF, TV and HR; the other parameters showed slight changes for doses of guanidine of 6400-12600 µg/kg. In the dose range of 50-800 µg/kg guanidine gave rise to slight and not significant hypotensive responses, and to a little reduction of dp/dt; for higher doses, guanidine caused, as far as systemic arterial pressure is concerned, biphasic responses of a slight entity (hypo-hypertension), coupled to a little increase in cardiac inotropism (dp/dt) and reduction of RF and TV, with no linear relationship with the doses employed.

Comparison with known active ingredients

In order to compare the activity of the compounds of the present invention to that of the products of the prior art, various doses of known anti-hypertensive, hypotensive and vasoactive drugs were administered to male Wistar rats under general anaesthesia by sodium thiopental (50 mg/kg, i.p.). The administration was effected through "rapid" i.v. route or through intravenous infusion (inf.) for a time of 5 minutes.

Responses in terms of changes in the systolic and diastolic systemic arterial pressure (ΔAP), of changes in heart rate (ΔHR) and in maximum increase in the rate of left ventricular isovolumetric pressure (Δdp/dt) are reported in the following Table 10.

TABLE 10

COMPOUND UNDER TEST		Δ AP (mmHg) SYSTOLIC	Δ AP (mmHg) DIASTOLIC	Δ HR (beats/min)	Δ dp/dt (mmHg/sec)
Guanetidine	(5 mg/kg, i.v.)	-27±5	-19±3	-18±6	- 863±160
Clonidine	(25 mcg/kg, inf.)	-15±4	- 9±1	-40±8	- 871± 74
Hexameton	(2.5 mg/kg, i.v.)	-44±2	-34±2	-48±9	-4040±812
Reserpine	(5 mg/kg, inf.)	-31±6	-24±5	-47±9	-1580±134
Papaverine	(2 mg/kg, i.v.)	-23±3	-18±3	-21±5	- 604±102
Adrenaline	(0.125 mcg/kg, i.v.)	+10±2	-10±0.5	+ 9±1	+1562±165
	(2 mcg/kg, i.v.)	+49±3	+39±2	+14±2	+3568±330
Noradrenaline	(1 mcg/kg, i.v.)	+44±3	+31±2	+14±4	+4314±415
Bradykinin	(0.75 mcg/kg, i.v.)	-18±3	-18±4	-11±2	- 830±124
Histamine	(5 mcg/kg, i.v.)	-30±3	-28±3	- 7±1	-1314±180
5-hydroxytriptamine	(5 mcg/kg,i.v.)	-37±3	-39±2	-12±3	-1410±167

n = 10 Mean values ± M.S.E. Average weight of the animals: 284 ± 17 g.

In table 10 the maximum responses are shown. The dose of each product is referred to the base, and the volumes injected were of 100 mcl in case of i.v. administration and 0.170 mcl total (0.9% sodium chloride solution) in case of infusion. The various parameters were evaluated in the same way as in the preceding experiments.

As it can be observed from the data of table 10, the hypotensive, anti-hypertensive or vasoactive agents which are largely and predominantly employed as therapeutic agents for primary and secondary arterial hypertension and for vascular disorders of a multiform nature (guanitidine, clonidine, reserpine, papaverine and so on) are usually characterized by general "depressive" effects on the various haemodynamic parameters.

The availability of a drug like guanido-I, which is capable of giving hypotensive responses while increasing heart rate and inotropism as well as respiratory rate (and consequently also the peripheral arterial flow) appears to be a clearly advantageous therapeutical aid (because of a number of obvious considerations) with respect to the drugs mentioned above.

The compound diguanido-II, characterized by a higher pharmacological power as to hypotensive effect with respect to guanido-I, shows a constant stimulating action on the heart inotropism. Moreover, at all dose levels it does not cause any reflected tachycardia, which is a typical and undesired effect of most antihypertensive drugs commercially available (for instance: those having arterial vasodilating action, all alpha-blocking agents but the selective alpha$_1$-blocking agent known as prazosine, hydrazino-phthalazine derivatives, and so on). The increase in the peripheral arterial flow (observed at lower doses) can be advantageous under ischemic conditions; said effect gives just slight changes in the cardiovascular and respiratory parameters.

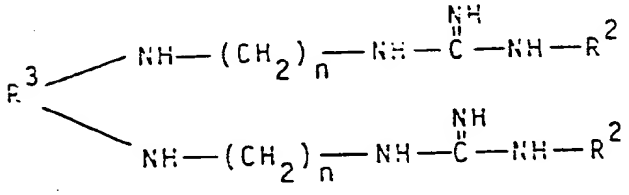
The employment of diguanido-II is thus advisable, for instance, in the treatment of arterial hypertension caused by an increase in the peripheral vascular resistance ("essential" hypertension, vasculosclerosis, and so on).

Claims

1. Guanidine derivatives of the formula:

$$R^1\text{-NH-(CH}_2\text{)}_n\text{-NH-C(=NH)-NH-}R^2 \quad (1)$$

wherein:
R^1 is hydrogen or optionally substituted cynnamoyl,
R^2 is hydrogen, alkyl or alkenyl, with the proviso that R^1 and R^2 cannot be both hydrogen, and
n is an integer from 1 to 8, or of the formula:



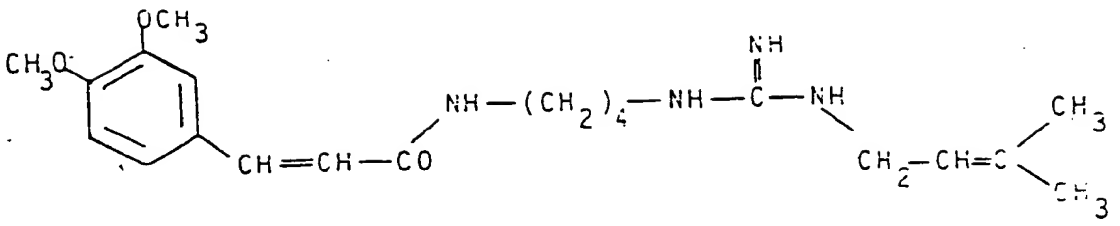
(2)

wherein:
R^3 is truxinoyl or truxilloyl, each optionally substituted, and
R^2 and n are as defined above.

2. Guanidine derivatives according to claim 1, wherein R^2 is alkyl or alkenyl of 1-5 carbon atoms.

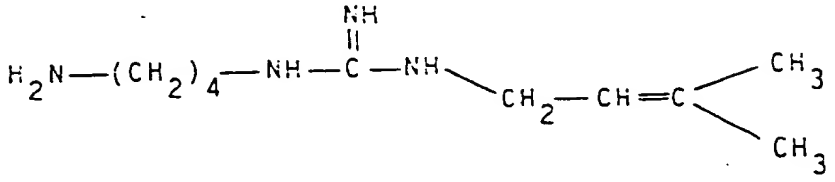
3. Guanidine derivatives according to claim 2, wherein R^2 is prenyl.

4. A guanidine derivative according to claim 3, of the formula:



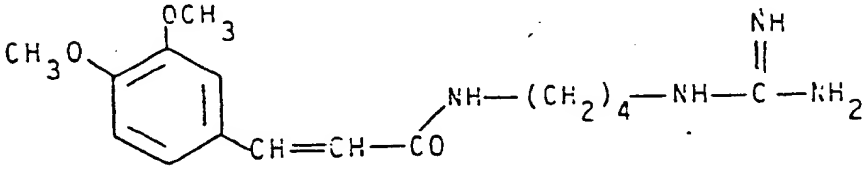
(3)

5. A guanidine derivative according to claim 3, of the formula:



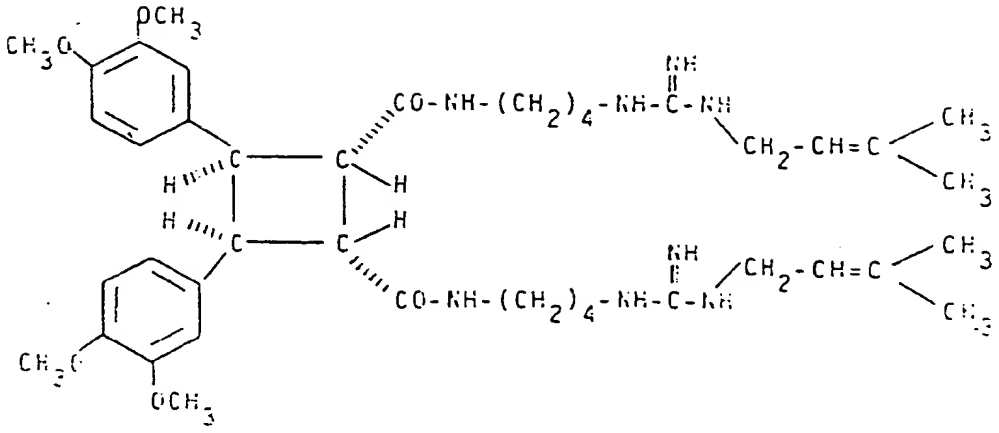
(4)

6. A guanidine derivative according to claim 1, of the formula:



(5)

7. A guanidine derivative according to claim 2, of the formula:



(6)

8. A pharmaceutical composition for treatment of hypertension, comprising and effective amount of one or more compounds as claimed in claims 1-7, in a pharmaceutically acceptable carrier.

9. A pharmaceutical composition for treatment of hypertension, containing the extract of a plant of the genus Verbesina in a pharmaceutically acceptable carrier.

10. A pharmaceutical composition according to claim 9, wherein said plant is the Verbesina caracasana.

11. A process for extracting and purifying compounds according to claims 1-7 from plant materials, said process being characterized in that is comprises the operation of:

a) treating the plant material made into small pieces or ground, with an alcoholic solvent for extraction;

b) removing said solvent in vacuo;

c) distributing the extraction residue between ethyl acetate and water;

d) subjecting the resulting water fraction to lyophilization (freeze-drying).

12. A process according to claim 11, wherein the ethyl acetate fraction resulting from said operation c) is further subjected to water extraction and the water extract so obtained is added to that resulting from said operation c).

13. A process for extracting and purifying compunds according to claims 1-7 from plant material, said process being characterized in that it comprises the operations of:

a) treating the plant material made into small pieces or ground with water at room temperature for performing the extraction;

b) subjecting the resulting water solution to lyophilization (freeze-drying).

14. A process according to each one of the preceding claims 11-13, wherein the lyophilized raw extract resulting from said operations d) or b) is suspended again in an anhydous alcoholic solvent and filtered, and then said solvent is removed by evaporation, so that a purified raw extract is obtained.

15. A process according to claim 14, wherein said operations consisting in re-dissolving the raw extract, filtering the suspension and evaporating the solvent are further repeated.

16. A process according to each one of the preceding claims 11-13, wherein said raw extract is subjected to separation of its components through a silica column by elution with chloroform containing increasing amounts of methanol.

17. A process according to each one of the preceding claims 11-15, wherein said raw extract is subjected to separation of its components by means of Sephadex LH-20 and elution with methanol.

18. A process according to each one of the preceding claims 11-17, wherein said plant material is obtained from Verbesina caracasana.

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 330 629**
A3

(12) EUROPEAN PATENT APPLICATION

(21) Application number: 89830067.8

(51) Int. Cl.5: C07C 129/12, A61K 31/155, A61K 31/16

(22) Date of filing: 17.02.89

(30) Priority: 24.02.88 IT 4766588

(43) Date of publication of application:
30.08.89 Bulletin 89/35

(84) Designated Contracting States:
AT BE CH DE ES FR GB GR IT LI LU NL SE

(88) Date of deferred publication of the search report:
31.10.90 Bulletin 90/44

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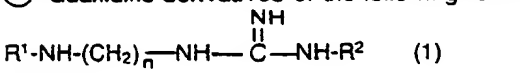
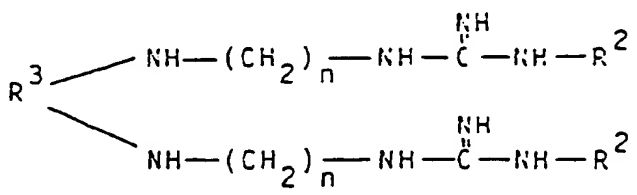
(54) Guanidine derivatives having hypotensive activity, composition containing them, and process for obtaining them.

(57) Guanidine derivatives of the following formulas (1) and (2):



(1)

wherein:
R^1 is hydrogen or optionally substituted cinnamoyl,
R^2 is hydrogen, alkyl or alkenyl, with the proviso that R^1 and R^2 cannot be both hydrogen,
n is an integer from 1 to 8, or:



(2)

wherein:
R^3 is truxinoyl or a truxilloyl each optionally substituted, and

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R^2 and n are as defined above;
pharmaceutical compositions containing such compounds and a process for their extraction and purification from plant material, in particular from Verbesina caracasana.

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Application Number

EP 89 83 0067

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
X	US-A-3 475 459 (STOESSL) * Examples 14-17; claims * ---	1	C 07 C 129/12 A 61 K 31/155 A 61 K 31/16
X	GB-A-2 186 563 (HENKEL CORP.) * Example 3 * ---	1,2	
X	CHEMICAL ABSTRACTS, vol. 94, no. 21, 25th May 1981, page 255, abstract no. 169612c, Columbus, Ohio, US; W.K. PAIK et al.: "Metabolism of Ng-monomethyl-L-arginine: formation of Ng-methylagmatine by Escherichia coli preparation", & CAN J. BIOCHEM. 1981, 59(2), 131-6 * Abstract * ---	1,2	
A	US-A-3 332 988 (MULL) * Column 2, lines 20-31; examples; claims * ---	1-10	
A	US-A-2 734 904 (BURTNER) * Examples; claims * ---	1-10	TECHNICAL FIELDS SEARCHED (Int. Cl.4)
A	GB-A- 985 635 (AB PHARMACIA) * Page 1, lines 69-76; examples; claims * ---	1-10	C 07 C 129/00
A	CH-A- 649 561 (RICHTER GEDEON VEGYESZETI GYAR R.T.) * Examples; claims * ---	1-10	
A	FR-E- 78 162 (CIBA) * Page 2, column 1, lines 21-36; examples; claims * --- -/-	1-10	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	25-07-1990	HELPS I.M.

CATEGORY OF CITED DOCUMENTS

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EUROPEAN SEARCH REPORT

Application Number

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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A	BE-A- 625 179 (SOCIETA FARMACEUTICI ITALIA) * Examples; claims *	1-10	

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
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